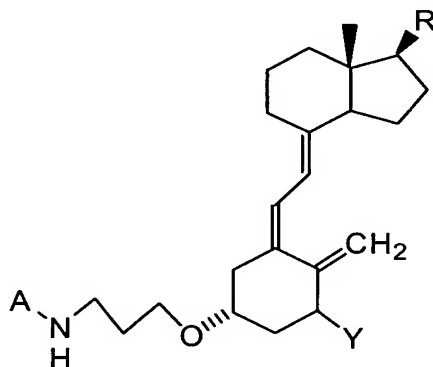


CLAIMS

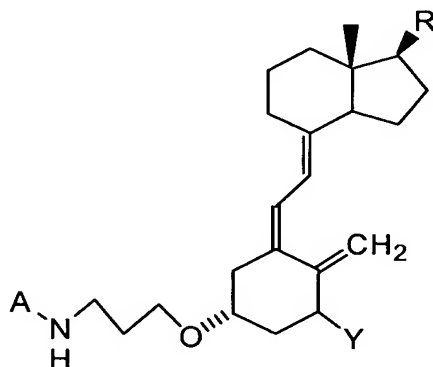
1. A method of measuring the amount of a 25-hydroxy- or
1 α ,25-dihydroxy vitamin D metabolite in a sample,
comprising measuring binding to or displacement from
a vitamin D binding protein of a vitamin D derivative
of the formula



wherein:

- R represents a 25-hydroxy side-group of vitamin D₂ or of vitamin D₃;
 - Y represents hydrogen or hydroxy;
 - A represents a functional group, coupled via a spacer group, which can be bound by a protein with high affinity;
- obtained by a method comprising:
- a) cyanoethylating the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;
 - b) adding lithium hydride and converting the 25-hydroxy group into the lithium alcoholate and subsequently reducing the nitrile group with lithium aluminium hydride; and
 - c) linking a spacer group together with a functional group A on the amino propylether side chain.

2. The method of claim 1, wherein the method is a competitive immunoassay, selected from the group consisting of radioimmunoassay, enzyme immunoassay, enzyme-linked immunosorbent assay, luminescence immunoassay and fluorescence immunoassay.
3. The method of claim 1, wherein the method is a sandwich immunoassay, selected from the group consisting of immuno radiometric assay, IEMA/EIA, immuno luminometric assay and immunofluorometric assay.
4. A kit for detection of 25-hydroxy- and $1\alpha,25$ -dihydroxy vitamin D metabolites comprising a standardized quantity of solid or a standardized solution of a vitamin D derivative of the formula



wherein:

- R represents a 25-hydroxy side-group of vitamin D_2 or of vitamin D_3 ;
 - Y represents hydrogen or hydroxy;
 - A represents a functional group, coupled via a spacer group, which can be bound by a protein with high affinity;
- wherein the vitamin D derivative is obtained by a

- method comprising:
- a) cyanoethylating the 3-hydroxy group of a vitamin D starting compound in the presence of potassium hydride and tertiary butanol;
 - 5 b) adding lithium hydride and converting the 25-hydroxy group into the lithium alcoholate and subsequently reducing the nitrile group with lithium aluminium hydride; and
 - 10 c) linking a spacer group together with a functional group A on the amino propylether side chain.
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- 15 5. The kit of claim 4, wherein the spacing group has the length of 0.9 to 1.5 nm.
 6. The kit of claim 4 wherein A is a biotin group and the spacing group has the length of 0.9 to 1.5 nm.
 - 20 7. The kit of claim 4 comprising a solid phase selected from the group consisting of a microtitration plate, another solid carrier, a microparticle, a polymeric material, and a cellulose.
 8. The kit of claim 7, in which the solid phase is a
25 microparticle comprising agarose.
 9. The kit of claim 7, in which the solid phase is a magnetic microparticle.
 10. The method of claim 1, wherein displacement of the
30 vitamin D derivative from a vitamin D binding protein is measured and the vitamin D derivative displaces a 25-hydroxy- or 1 α ,25-dihydroxy vitamin D metabolite from the vitamin D binding protein with a displacement efficiency of approximately 1.

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